REMARKS

Claims 1-5 remain pending in the application. Applicants respectfully request reconsideration of the application in view of the following remarks.

Rejection Under 35 U.S.C. §101

The PTO maintains its rejection of Claims 1-5 under 35 U.S.C. § 101 as lacking a substantial asserted utility or a well-established utility for the reasons set forth in the previous Office Actions. The PTO asserts that one skilled in the art would not know how to use the claimed invention. According to the PTO, "there is insufficient information or experimental data presented on whether the polypeptide or the antibodies binding such of the present invention can serve as a reliable diagnostic marker for distinguishing normal kidney from kidney tumor." *Final Office Action* at 5. The PTO relies on Hu *et al.* and other references for the propositions that the literature cautions researchers against drawing conclusions based on small changes in transcript expression levels between normal and cancerous tissue, and that what is often seen is a lack of correlation between DNA expression and increased peptide levels. The PTO argues that further research is required to determine whether the PRO1069 polypeptide is differentially expressed, making the asserted utility for the claimed polypeptides not substantial.

The PTO cites Hu and LaBaer as teaching that small changes in mRNA level may not be biologically meaningful, and maintains that mRNA levels do not correlate with polypeptide levels. In support of this position, the PTO also cites Alberts [a] and Alberts [b], Lewin, Zhigang, Meric, Haynes et al., Gygi et al., Greenbaum et al., Jang, Lian et al., Fessler et al., Hanash [a] and Hanash [b], Winstead and Irving.

With respect to the previously submitted Exhibits in support of Applicants' position that changes in mRNA levels are correlated with changes in the levels of the encoded polypeptides, the PTO asserts that, with the exception of the Orntoft reference, each of the references submitted are directed to a single gene, or a small number of genes, and are not persuasive in view of comprehensive studies where significantly larger numbers of transcripts and proteins were examined, such as the newly cited references by Nagaraja, Waghray and Sagynaliev. In addition, the PTO indicates that Orntoft is not persuasive because the authors compared the mRNA and protein levels of about 40 well-resolved and focused abundant proteins with known chromosomal

locations while the instant specification does not teach whether or not PRO1069 is a "well focused abundant" protein with a known chromosomal location.

With respect to previously submitted Exhibits 17-24, the PTO maintains that, with the exception of the Futcher reference, these references are all directed to analysis of single genes, or a small group of genes. According to the PTO, these references do not demonstrate trends found across proteins in general. The PTO asserts that more comprehensive analyses like Haynes, Gygi, Chen et al. or Futcher more accurately describe general trends.

With regard to the Lian and Fessler references, the PTO asserts that these references support the position that changes in mRNA do not necessarily reflect changes in protein level because, in both studies, the researchers found a larger number of transcripts that were differentially expressed than proteins that were differentially expressed.

While the PTO acknowledges the teachings of Alberts [a], [b] and Lewin that initiation of transcription is the most common point for a cell to regulate the gene expression, they assert that these references demonstrate that there are a number of other controls that can act later in the pathway from RNA to protein to modulate the amount of protein that is made. The PTO asserts that the Meric reference is also consistent with this position. In addition, the PTO cites Nagaraja, Waghray, and Sagynaliev as showing that mRNA levels are not necessarily predictive of protein levels.

Applicants incorporate by reference their previously submitted arguments, and for the reasons of record assert that the specification contains a disclosure of utility and therefore must be taken as sufficient to satisfy the utility requirement of 35 U.S.C. § 101. Applicants also submit that for reasons of record, the PTO has not met its burden of providing evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility. However even if the PTO has met its initial burden, Applicants' rebuttal evidence previously submitted and additional evidence submitted herewith is sufficient to prove that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true. As stated previously, Applicants' evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. **The standard is not absolute certainty.**

Substantial Utility

Summary of Applicants' Arguments and the PTO's Response

Applicants' asserted utility rests on the following argument:

1. Applicants have provided reliable evidence that mRNA for the PRO1069 polypeptide is expressed at least two-fold higher in normal kidney compared to kidney tumor tissue;

2. Applicants assert that it is well-established in the art that a change in the level of mRNA for a particular protein, *e.g.* a decrease, generally leads to a corresponding change in the level of the encoded protein, *e.g.* a decrease;

3. Given the differential expression of the PRO1069 mRNA in kidney tumor, it is more likely than not that the PRO1069 polypeptide is also differentially expressed in kidney tumor, making the PRO1069 polypeptide and the claimed antibodies useful as diagnostic tools, alone or in combination with other diagnostic tools.

Applicants understand the PTO to be making two arguments in response to Applicants' asserted utility:

1. The PTO challenges the reliability of the evidence reported in Example 18, stating that Hu et al. and LaBaer et al. caution researchers from drawing conclusions based on small changes in transcript expression levels between normal and diseased tissue;

2. The PTO cites Alberts [a] and Alberts [b], Lewin, Zhigang, Meric, Haynes et al., Gygi et al., Greenbaum et al., Jang, Lian et al., Fessler et al., Hanash [a] and Hanash [b], Winstead and Irving, Chen et al., Nagaraja, Waghray, and Sagynaliev to support its position that one of skill in the art would not know if the disclosed change in PRO1069 mRNA transcripts is associated with a corresponding change in the level of PRO1069 protein.

The PTO has Concluded that the data in Example 18 are Sufficient to Establish the Utility of the Claimed Invention

As an initial matter, Applicants point out that in other applications filed by Applicants that rely *on data from the exact same disclosure, Example 18*, and in which Applicants have submitted *substantially the same references* in support of their asserted utility, the PTO has concluded that:

"[b]ased on the totality of evidence of record, **one of skill in the art would find it more likely than not that an increase in message as measured by RTPCR would be predictive of an**

increase in protein expression levels, absent evidence to the contrary. Therefore, the data presented in Example 18, which demonstrates differential expression of nucleic acids encoding PRO1180, also supports a conclusion of differential expression of PRO1180 polypeptide. Therefore, one of ordinary skill in the art would be able to use the PRO1180 polypeptide diagnostically for distinguishing normal kidney and rectal tumor tissues compared to kidney tumor and normal rectal tissue, as asserted by Applicant." See *Examiners Reasons for Allowance* in pending Application No. 10/063,529. See also *Examiners Reasons for Allowance* in Application No. 10/063,530, No. 10/063,524, No. 10/063,582, and No. 10/063,583, all of which conclude that the data presented in Example 18, which demonstrate differential expression of the nucleic acids encoding certain PRO polypeptides, also support a conclusion of differential expression of the PRO polypeptides, making the claimed PRO polypeptides and antibodies that bind the PRO polypeptides useful for diagnostic purposes.

Applicants therefore request that the Examiner recognize the utility of the claimed invention, supported by the data presented in Example 18 and Applicants numerous cited references, as was done in the other applications referenced above.

The Data Reporting Differential Expression of PRO1069 mRNA is Sufficient to Provide Utility for the mRNA as a Diagnostic Tool

Applicants next address the PTO's argument that the evidence of differential expression of the gene encoding the PRO1069 polypeptide in kidney tumor is insufficient, and that the literature cautions against drawing conclusions based on small changes in transcript expression levels between normal and cancerous tissue.

Applicants submit that the PTO's position that additional details regarding Example 18 are required to establish utility for the claimed polypeptides is beyond that required under 35 U.S.C. §101. Applicants' statement of utility is presumed to be true, and further evidence to establish utility should not be required. *See In re Langer*, 503 F.2d at 1391, 183 USPQ at 297; *In re Malachowski*, 530 F.2d 1402, 1404, 189 USPQ 432, 435 (CCPA 1976); *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995); *M.P.E.P.* §2107.02 (III). Requests for additional evidence should be imposed rarely, such as only when a statement is incredible in the light of the knowledge of the art, or factually misleading. *In re Citron*, 325 F.2d 248, 139 USPQ 516 (CCPA

1963); *M.P.E.P.* §2107.02 (V). In addition, as stated above, the standard for establishing a utility is a low one, and statistical certainty is not required:

> [T]he applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." **Nor must the applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty**. Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. M.P.E.P. at § 2107.02, part VII (2004) (underline emphasis in original, bold emphasis added, internal citations omitted).

The Federal Circuit has clearly rejected a requirement that evidence of utility be numerically precise or statistically significant. As previously argued, in *Nelson v. Bowler*, 626 F.2d 853, 206 U.S.P.Q. 881 (C.C.P.A. 1980), the issue in the interference was whether Nelson had shown at least one utility for the compounds at issue to establish an actual reduction to practice. *Id.* at 855. The Appellants relied on two tests to prove practical utility: an *in vivo* rat blood pressure (BP) test and an *in vitro* gerbil colon smooth muscle stimulation (GC-SMS) test. In the BP test, responses were categorized as either a depressor (lowering) effect or a pressor (elevating) effect. *Id.* The Board held that Nelson had not shown adequate proof of practical utility, characterizing the tests as "rough screens, uncorrelated with actual utility." *Id.* at 856.

On appeal the C.C.P.A. reversed, holding that the Board "erred in not recognizing that tests evidencing pharmacological activity may manifest a practical utility even though they may not establish a specific therapeutic use." *Id.*

This case is of importance because the Court rejected the notion that the testing must be statistically significant to support a practical utility. *Nelson*, 626 F.2d at 857. Likewise, qualitative characterizations of a test compound as either increasing or decreasing blood pressure was acceptable. *Id.* at 855 (stating that responses were categorized as either a depressor (lowering) effect or a pressor (elevating) effect). This is the same as the data in Example 18 relied on by Applicants, where the change in mRNA levels is described as "more highly expressed." The PTO's requirement that Applicants provide numerical precision and statistical certainty to establish utility is contrary to established standards for utility. Thus, these arguments do not support the PTO's position as they do not lead one skilled in the art to question Applicants' asserted utility.

The PTO dismisses the results of Example 18 "[i]n view of the limited disclosure in the instant case, lack of disclosure of the 'fold amplification' that was used to determine whether a higher expression, i.e., 'more highly expressed' was significant, lack of the statistical analysis, and lack of establishment of a correlative link between gene expression and protein level or a causal link between mRNA expression and kidney tumour..." *Final Office Action* at 8-9. Thus, the PTO has set a heightened requirement for Applicants to demonstrate utility based on the disclosed differential expression. Moreover, the PTO fails to support this heightened requirement with any evidence whatsoever. The PTO provides no evidence or findings of facts to suggest that one skilled in the art would doubt Applicants' disclosed differential expression. Based on the complete failure to present any evidence to bring into question Applicants' disclosed differential expression, Applicants submit that the PTO's requirement for statistically significant evidence is improper and insufficient to overcome Applicants' presumption of utility.

With respect to the Hu and LaBaer references cited by the Examiner, Applicants continue to maintain that these references do not contradict Applicants' position, because they focus on the role of polypeptides in cancer, and that whether or not the PRO1069 polypeptide is the causative agent of cancer, the claimed polypeptides are useful as diagnostic agents. In addition, as previously noted, Applicants' are not relying on microarray data as discussed in Hu and LaBaer. Instead, they are relying on a more accurate and reliable method of assessing changes in mRNA level, namely quantitative PCR analysis. Applicants again direct the Examiner's attention to the article by Kuo *et al.*, (Proteomics 5(4):894-906 (2005), previously submitted, which confirmed that in contrast to the results obtained using microarrays, more reliable and sensitive analyses, such as reverse transcriptase polymerase chain reaction [RT-PCR], Western blotting and functional assays, demonstrated a good correlation between mRNA and protein expression. As previously noted, even if accurate, Hu and LaBaer's statements regarding microarray studies are not relevant to the instant application which does not rely on microarray data. Instead, the data in Example 18 were obtained using RT-PCR.

Applicants respectfully submit that one of skill in the art would not accept that the PTO has established a basis to doubt Applicants' asserted utility. Those of skill in the art recognize that RT-PCR is a more accurate and reliable technique than microarrays (see, e.g., Kuo *et al.*,). Therefore, it would be readily apparent to one skilled in the art that opinions regarding data from

high-throughput techniques such as microarrays are simply not relevant to Applicants' RT-PCR data, and are not a reason to doubt the truth of Applicants' asserted utility. Thus, even if accurate, a point which Applicants do not concede, Hu's and LaBaer's opinions regarding microarray studies are not relevant to the utility of the instant application which does not rely on microarray data.

Applicants emphasize that they are not asserting that microarray data are not reliable, merely that Applicants are using a method that is recognized by those of skill in the art as more reliable and sensitive.

In conclusion, Applicants submit that the evidence reported in Example 18, supported by the first Grimaldi Declaration, establish that there is at least a two-fold difference in PRO1069 mRNA in kidney tumor. Therefore, the only issue which remains is whether the data in Example 18 regarding differential expression of the PRO1069 mRNA are reasonably correlated with differential expression of the PRO1069 polypeptide such that the PRO1069 polypeptides and claimed antibodies have utility as diagnostic tools as well. As discussed above, the PTO has concluded in several other applications that the data in Example 18 are sufficient to establish the utility of the claimed invention. In addition, as discussed below, Applicants' overwhelming evidence is more than sufficient to establish that changes in mRNA level lead to corresponding changes in protein level.

The PTO's Evidence is Not Relevant to Determining Whether a Change in mRNA Level for a Particular Gene leads to a Corresponding Change in the Level of the Encoded Protein

Applicants turn next to the second portion of their argument in support of their asserted utility – that it is well-established in the art that a change in the level of mRNA encoding a particular protein generally leads to a corresponding change in the level of the encoded protein; given Applicants' evidence of differential expression of the mRNA for the PRO1069 polypeptide in kidney tumor, it is likely that the PRO1069 polypeptide is also differentially expressed; and proteins differentially expressed in certain tumors and the antibodies that bind said proteins have utility as diagnostic tools.

In response to Applicants' assertion, the PTO relies on several references including Haynes, Gygi, Chen, Futcher, Hanash [a] and Hanash [b]. In addition, while the PTO

acknowledges that the initiation of transcription is the most common point for a cell to regulate gene expression, the PTO cites Alberts [a], [b] Lewin, and Meric, which were submitted by the Applicants in support of their position, as supporting the conclusion that there are many factors that determine translation efficiency for a given transcript or the half-life of the encoded protein.

Applicants have previously discussed at length why the cited references do not contradict Applicants' position that, in general, differential mRNA expression correlates with differential expression of the encoded polypeptide. Applicants incorporate by reference the previous arguments, and will not repeat them here.

The PTO newly cites Lian et al. and Fessler et al. as showing poor correlation between mRNA expression and protein expressions (see *Final Office Action* at 12.) In Lian, the authors looked at the mRNA and protein levels of genes in a derived promyelocytic mouse cell-line during differentiation of the cells from a promyelocytic stage of development to mature neutrophils following treatment with retinoic acid. *Lian* at Abstract. The level of mRNA expression was measured using 3'-end differential display (DD) and oligonucleotide chip array hybridization, and protein levels were qualitatively assessed following 2-dimensional gel electrophoresis. *Id.* at Abstract, Table 6.

Lian *et al.* used DD and array hybridization to examine the expression of genes 0, 24, 48 and 72 hours after treatment with retinoic acid. *Id.* at 515, col. 1, ¶ 2. Using this information, the authors constructed a database of mRNA level changes during differentiation of the cell line. *Id.* at 518, col. 2, ¶ 2. Lian *et al.* also examined protein expression at 0 and 72 hours after retinoic acid treatment. Lian reports that they were able to identify 28 proteins which they considered differentially expressed. *Id.* at 521, Fig. 5. Of those 28, only 18 had corresponding gene expression information in the database, and only 13 had measurable levels of mRNA expression. *Id.* at 521, Table 6. The authors then compared the qualitative protein level from the 2-D electrophoresis gel to the corresponding mRNA level, and reported that only 4 genes of the 18 present in the database had expression levels which were consistent with protein levels. *Id.* at 521, col. 1. The authors note that "[n]one of these was on the list of genes that were differentially expressed significantly (5-fold or greater change by array or 2-fold or greater change by DD)." *Id.* at 521, bridge paragraph (emphasis added). Based on these data, the authors conclude "[f]or protein levels based on estimated intensity of Coomassie dye staining in

2DE, there was poor correlation between changes in mRNA levels and estimated protein levels." *Id.* at 522, col. 2, ¶ 2.

These results are not contrary to Applicants' assertion. Applicants emphasize that they are asserting that measurably differential mRNA levels generally lead to corresponding differential levels of protein expression, not that differential protein levels can be used to predict differential mRNA levels. Based on the authors' criteria, mRNA levels were significantly differentially expressed if they were at least 5-fold different when measured using a microchip array, or 2-fold different when using the more sensitive PCR–based cDNA differential display method (DD). Of the 28 proteins listed in Table 6, only one has an mRNA level measured by microarray which is differentially expressed according to the authors (spot 7: melanoma X-actin, which mRNA changed from 2539 to 341.3, and protein changed from 1 to 3). None of the other mRNAs listed in Table 6 show a significant change in expression level when using the criteria established by the authors for the less sensitive microarray technique.

There is also one gene in Table 6 whose expression was measured by the more sensitive technique of DD, and its level increased from a qualitative value of 0 to 2, a more than 2-fold increase (spot 2: actin, gamma, cytoplasmic). This increase in mRNA was accompanied by a corresponding increase in protein level, from 3 to 6.

Therefore, although the authors characterize the mRNA and protein levels as having a "poor correlation," this does not reflect a lack of a correlation between differential mRNA levels and corresponding differential protein levels. Only two genes meet the authors' criteria for differentially expressed mRNA level, and of those, one apparently shows a corresponding differential protein level and one does not. *Id.* at 521, Table 6. Thus, there is no basis for the PTO to cite this reference as a "comprehensive study" that weighs against Applicants' asserted utility.

The PTO also cites a publication by Fessler *et al.* Fessler is not contrary to Applicants' asserted utility, and actually supports Applicants' assertion that differential levels of mRNA for a particular protein generally leads to corresponding differential levels of the encoded protein. As noted above, Applicants make no assertions regarding differential protein levels when mRNA levels are unchanged, nor does evidence of differential protein levels when mRNA levels are unchanged have any relevance to Applicants' asserted utility.

Fessler *et al.* studied differential gene transcription and protein expression in neutrophil (PMN) following lipopolysaccharide (LPS) exposure. Fessler *et al.* lists in Table VIII a comparison of the differential levels of mRNA for 13 up-regulated proteins and 5 down-regulated proteins. Of 13 up-regulated proteins, Fessler *et al.* report differential mRNA levels in only 3 such proteins. For these 3, mRNA levels are increased in 2 and decreased in the third. Thus, in 2 out of 3 cases, an increase in mRNA correlated with an increase in protein levels. Of 5 down-regulated proteins, differential mRNA levels are reported for 3 such proteins. In all 3, mRNA levels also are decreased. Thus, in every case, a decrease in mRNA correlated with a decrease in protein levels. The data in which differential mRNA levels are reported are the only data that are relevant to Applicants' asserted utilities. In 5 of the 6 cases for which differential mRNA levels are reported, the differential levels of mRNA corresponds to the differential levels of the protein. This is consistent with Applicants assertion that differential levels of mRNA for a particular protein generally leads to corresponding differential levels of the encoded protein.

Six proteins listed in Table VIII relate to instances of differential protein levels accompanied by unchanged mRNA levels. Applicants make no assertion as to whether or not differential protein levels "necessarily" correlate with differential mRNA levels. This evidence has no relevance to Applicants' assertion that differential mRNA levels lead to corresponding differential protein levels, since Applicants are not asserting that differential mRNA levels are the only cause of differential protein levels. In accounting for these results, Fessler *et al.* explains that LPS has post-transcriptional activity that can influence protein levels (Fessler *et al.* at 31300, right column). Nothing in these results by Fessler *et al.* suggests that differential levels of mRNA for a particular protein do not generally lead to corresponding differential levels of the encoded protein. Accordingly, these results are not contrary to Applicants' assertions.

In the final 6 instances listed in Table VIII, protein levels changed while mRNA was noted as "absent." This evidence also has no relevance to Applicants' assertion that differential mRNA levels causes corresponding differential protein levels. By virtue of being "absent," it is not possible to tell whether mRNA levels were increased or decreased in PMN upon contact with LPS. Regarding these instances, Fessler *et al.* explains that LPS may have post-translational activity that can result in increased protein stability (Fessler at 31300, right column). Nothing in these results by Fessler *et al.* suggests that differential levels of mRNA for a particular protein do

not generally lead to corresponding differential levels of the encoded protein. Accordingly, these results also are not contrary to Applicants' assertions.

Thus, Fessler's results suggest that LPS has a transcriptional activity that can cause differential mRNA levels which correlate with differential protein levels, and that LPS also has post-transcriptional activity that can cause differential protein levels that are not related to differential mRNA levels. Accordingly, Fessler's results are consistent with Applicants' assertion that differential levels of mRNA for a particular protein generally leads to corresponding differential levels of the encoded protein.

Even if Fessler's results had shown that differential levels of mRNA did not generally lead to corresponding differential levels of the encoded protein, which they did not, the accuracy of Fessler's results is uncertain. Fessler *et al.* states that there were "limitations" to the results reported. These limitations included: possible artifactual transcript-protein discordance due to a 4 hour delay in harvesting after LPS exposure; uncertain post-incubation but pre-electrophoresis effects on protein synthesis, degranulation and exocytosis; and limited ability to quantitate protein amounts using Coomassie Blue. (Fessler *et al.* at 31301, left column). Fessler *et al.* exemplifies one such spurious result, in which there was a disparity between observed increase in cytokine mRNA, but an absence of detected cytokine proteins, which, as Fessler *et al.* explains, "reflects their removal in the post-LPS incubation wash." (Fessler *et al.* at 31297, right column). Thus, Fessler *et al.* acknowledges "limitations" to the conclusion that, for some genes, transcript levels did not coincide well with corresponding protein levels, leaving it uncertain the extent to which actual differential protein levels differed from mRNA levels when neutrophils were exposed to LPS.

Fessler *et al.* therefore represents a teaching that LPS might cause differential transcription that correlates with differential protein levels, and might also result in post-transcriptional affects on protein levels when mRNA levels are unchanged. Accordingly, Fessler *et al.* is not contrary to Applicants' asserted utility.

The PTO also newly cites references by Nagaraja *et al.* (Oncogene, (2006) 25:2328-38), Waghray *et al.* (Proteomics, (2001) 1:1327-38) and Sagynaliev *et al.* (Proteomics, (2005) 5:3066-78) as support for the argument that "mRNA levels are not necessarily predictive of protein

levels" and that "this is true even when there is a change in the mRNA level." *Final Office Action* at 16.

The PTO argues that in Nagaraja *et al.*, researchers observed that there were fewer changes observed in protein abundance as compared to transcript abundance between various malignant and normal breast cell lines and that "[t]he comparison of transcript profiles with proteomic profiles demonstrated that altered proteins were not always represented in the microarray designated profiles and *vice versa*". *Id.* at 17. The PTO sees these observations as support for its contention that mRNA levels are not predictive of protein levels, even when considering the effect of changes in mRNA levels on protein levels. However, a careful examination of Nagaraja *et al.* shows that the reference does not contain evidence that supports the PTO's position.

Nagajara and colleagues analyzed the transcriptosomes and proteosomes of normal and malignant breast cell lines. In the studies of the transcriptosomes of these cell lines, the gene chips used in the microarray analysis could detect 18,400 gene or gene variants. Nagajara *et al.* reported over 1000 genes that had a two-fold or greater differential level of expression between the various cell lines studied. The researchers distingushed between differentially expressed transcripts that were upregulated and those that were downregulated, comparing the normal cell line to the malignant ones and the malignant ones to each other (Figure 5, pg. 2332).

However, in their proteosome studies, the researchers used techniques that were far less sensitive and only able to detect a small number of proteins: "Typically, > 300 protein spots could be visualized in silver-stained gels, and there were far fewer protein spots in gels that were stained with Coomassie blue" (pg. 2332). While the gene chips used by the researchers in their transcriptosome work could detect 18,400 gene and gene variant transcripts, the proteosome analysis techniques used by the researchers could only detect a much smaller number of proteins. Evidently, the protein analysis techniques used were not sensitive enough to detect any but the most abundant proteins. As a result, the total number of proteins detected in the most sensitive protein gel used ("> 300") was only 1/3 of the total number of transcripts found to be differentially expressed and only about 17% of the total number of transcripts that could be identified by microarray analysis. The proteins detected do not represent a random, representative sample of proteins from the cells; instead, they actually represent a sample of only

the most highly expressed and abundant proteins. Additionally, the proteins selected for identification from the gels were only those proteins that were either *upregulated* or solely detected in the malignant cell lines, as compared to the normal cell line. Proteins that were downregulated in the malignant cell lines, only expressed in the normal cell line or differentially expressed between the malignant cell lines were not studied in the proteosome analysis.

Nagaraja's experimental methods and results are not contrary to Applicants' assertions. Nagaraja's "comprehensive study" looked at no more than 25 mRNA/protein pairs. The total number of proteins detected in the most sensitive protein gel was about 300, which was less than 2% of the total number of transcripts that were identified by Nagaraja's microarray analysis. The proteins selected by Nagaraja as differentially expressed were only those proteins that were solely detected in the malignant cell lines, as compared to the normal cell line. Proteins that were detected in the normal cell line, whether upregulated, downregulated, or absent in the malignant cell lines relative to the normal cells lines, were omitted from the proteosome analysis. In the end, only 25 proteins were examined, which is about 2.5% of the number of differentially expressed transcripts detected. Even then, Nagaraja does not indicate whether or not corresponding transcripts were detected for each of these 25 proteins. Thus, Nagaraja looked at 25 or fewer mRNA/protein pairs.

Nagaraja's experimental methods and results do not teach even a single instance in which differentially expressed mRNA did not have a similarly differentially expressed encoded polypeptide. Nagaraja may very well have detected some differentially expressed proteins that were not associated with differentially expressed mRNAs. Such a result is not contrary to Applicants' assertions that differential mRNA levels typically correspond to differential levels of the encoded polypeptide. Evidence in Nagaraja of differentially expressed protein when mRNA is not differentially expressed cannot lead to the conclusion that differential mRNA levels fail to lead to corresponding differential protein levels. Accordingly, these results cannot be construed as contrary to Applicants' assertions.

Due to the difference between the techniques used and the strategies employed by the researchers in this study, the data from the transcriptosome and proteosome studies cannot be reliably compared to one another. The transcriptosome studies examined 18,400 transcripts and variants and uncovered thousands of differentially expressed transcripts, both upregulated and

downregulated. The proteosome studies only detected around 300 of the most abundant proteins in the cell lines. The researchers only selected proteins that were upregulated or solely expressed in malignant cells for study. Thus, genes expressed at a relatively low rate or gene products that are relatively less abundant were included in the transcriptosome study but excluded from the proteosome study. Additionally, different criteria were used for defining altered expression of transcripts than were used to select proteins for identification. The criteria for picking a protein for study (only those upregulated by two-fold or greater, or solely expressed, in malignant cells) was narrower than the criteria for examining differentially expressed transcripts (any transcript with a two-fold or greater upregulation or downregulation between any combination of two of the three cell lines examined). Thus, the population of genes examined in the proteosome experiments represents a small, non-random subset of the population examined in the transcriptosome experiment, both in terms of the total population of transcripts or gene products uncovered by the experiments and in terms of those particular transcripts or gene products that were identified as altered in expression. Because the genes analyzed in the proteosome experiments represent neither a similar set nor a representative, randomly selected subset of the genes analyzed in the transcriptosome experiments, no valid conclusions can be drawn by comparing the results from the two types of experiments to one another.

The PTO cites several sentences from Nagaraja *et al.* in support of its argument. Specifically, the PTO cites:

> "...the proteomic profiles indicated altered abundance of few proteins as compared to transcript profiles...";
>
> "The comparison of transcript profiles with proteomic profiles demonstrated that altered proteins were not always represented in the microarray designated profiles and *vice versa*"; and
>
> "As dictated by post-transcriptional regulation, protein profiles showed far fewer changes as compared to transcript profiles."

However, as the above analysis of experimental techniques and design reveals, transcriptosome and proteosome study data cannot be compared to each other to draw conclusions about the relationship between mRNA levels and protein levels. This is due to a wide difference in technique sensitivity, which lead to the examination of drastically different portions of the total

gene transcript or product populations, and an incompatible difference in the definition of altered expression for transcripts and proteins. Nagajara *et al.* found that the proteomic profiles had fewer proteins with an altered abundance as compared to the transcriptosome profiles and that the same genes with altered expression patterns were not always found in both proteomic and transcriptosomic profiles. However, from the observations made during analysis of the results of transcriptosome and proteosome research, Nagajara *et al.* drew no conclusions as to the relationship between mRNA levels and protein levels. All comments made by the authors are entirely consistent with conclusions of Sagynaliev, discussed *infra*: that there is a significant need to standardize the scientific methods of collecting, storing, retrieving and analyzing samples, as well as the querying of genetic expression data obtained through a variety of techniques. By pointing out the differences between the proteosome and transcriptosome studies, the authors were not implying that there was doubt about the relationship between mRNA levels and protein levels. Rather, they were pointing out the unreliability of any conclusions that could be drawn from comparisons between studies of differential transcriptosomes and studies of differential proteosomes.

Regarding the third statement relied upon by the PTO, the conclusions of Nagaraja *et al.* about *post-transcriptional* regulation are based on studies of a cell line that was genetically engineered in the laboratory to eliminate particular transcripts through the use of anti-sense sequence technology. In these experiments, the authors deliberately reduced certain particular transcripts and then looked at the effects on cell cultures and proteosomes at one time point (when cultures were 70-80% confluent). As explained in greater detail under Waghray *et al. infra*, sudden changes and manipulations of transcript profiles can lead to wildly fluctuating levels of gene product within cells. Additionally, the amount of time that is needed to see the difference in gene product levels caused by changes and manipulations of transcript levels can vary widely from gene to gene, from hours to multiple days. Thus, examination of only one timepoint is insufficient to draw conclusions about the effects of dynamic changes and manipulations of transcript levels on protein abundance and is not relevant to the correlation between steady-state levels of mRNA and gene products. Furthermore, the authors are not certain about how the introduction of the antisense constructs is actually working to reduce the presence of the particular transcripts in question: "the antisense constructs... *appeared* to work

as siRNAs..." (pg. 2335, emphasis added). In any case, laboratory data from cells genetically manipulated with non-native, unnaturally occurring sequences, which were packaged into expression vectors with foreign sequence elements and produced effects from uncertain subcellular mechanisms, has no relevance to Applicants' instant invention.

The PTO cites particular observations made in Waghray *et al.* to support its argument that mRNA levels are not necessarily predictive of protein levels, even when there is a change in mRNA levels. Waghray *et al.* looked at transcriptosomal and proteosomal changes in an androgen-sensitive prostate cancer cell line after the cells were treated with dihydrotestosterone (DHT). Out of 16,570 genes, the authors found 351 transcripts that were differentially expressed in the stimulated cells. The authors also identified 44 proteins, out of 1031 spots on protein gels, that were upregulated or downregulated in stimulated cells. Hence, Waghray *et al.* found that over 4% (44/1031) of the proteins isolated from the cells were differentially expressed while only 2% (352/16,570) of the transcripts were differentially expressed.

The PTO posits that if changes in protein generally reflected mRNA changes, one would only expect to see 2% of the proteins differentially expressed, i.e. 22 out of 1031 proteins, instead of the observed 44 proteins. However, Applicants make no assertion that differential protein levels always are accompanied by differential mRNA levels. The possibility that additional factors beyond differential mRNA levels also can lead to differential protein levels does not imply that differentially expressed mRNA do not typically have a corresponding differentially expressed encoded polypeptide.

The PTO's conclusion requires that the set of 1,031 proteins found in the protein gels be a proportional, representative, randomized subset of the 16,570 genes found in the analysis of the transcripts. It is clear that the 1031 proteins found represent only a small subset of the 16,570 transcripts examined. The authors stated that "[a] relatively small set of genes could be analyzed at the protein level, largely due to the limited sensitivity of 2-D PAGE" (pg. 1337, emphasis added). Thus, similar to the results of Nagaraja *et al.* discussed *supra*, proteins found through the use of protein gels represent only the most abundant proteins of the cell, whereas the transcripts identified included transcripts of many proteins not abundant enough to be found via protein gels. Because the set of proteins identified do not represent a randomized subset of the transcripts

identified, one cannot draw conclusions regarding the general relationship between changes in mRNA levels and changes in protein levels based on a comparison of these data.

The PTO also cites an additional statement by Waghray *et al.* in support of its argument that mRNA levels are not necessarily predictive of protein levels, even when there is a change in mRNA levels. Waghray *et al.* found that corresponding SAGE (sequence analysis) data were available for a number of the proteins identified as differentially expressed and stated that "remarkably, for most of the proteins identified, there was no appreciable concordant change at the RNA level (Table 4)." *Final Office Action* at 17. The PTO concludes from this statement that the data presented by Waghray *et al.* support its argument against a correlation between mRNA levels and protein levels.

However, further analysis of the data collected in these experiments shows that such a conclusion cannot be drawn from the data. The experiments of Waghray *et al.* that produced the data shown in Table 4 involve hormonally stimulating cells for 24 hours; determining mRNA levels in the cells; and, 48 hours after determining mRNA levels, determining protein levels, for specific mRNA/protein product pairs. The authors measured mRNA levels twice, before stimulating with DHT and after stimulating with DHT for 24 hours (24 hours post-treatment). They also measured protein concentrations twice, before stimulating with DHT and at 72 hours post-treatment. The second measurement of protein levels therefore occurred 48 hours after DHT was removed from the culture media. Thus, the experiment involved creating a dynamic and changing environment for cells and the measurement of the effects of this changing environment at only one timepoint. Additionally, the timepoints used for measuring the effects on mRNA levels and protein levels were 48 hours apart.

Examining the two timepoints for particular genes, the authors stated that there was not appreciable concordant change at the RNA level for most of the proteins whose concentrations were affected by DHT treatment. However, the differential expression of mRNA at 24 hours and of protein at 72 hours does not reveal the complete picture of the effects of DHT treatment on the cells. The authors noted that the dynamic conditions of the experiments created fluctuating levels of both mRNA and protein over time (pg. 1337). They decided to examine the kinetics of mRNA and protein levels for two proteins affected by DHT treatment, PSA and clusterin (Fig. 1C on pg. 1334). PSA is known to be an androgen-regulated gene and the authors had been

surprised to see only a 1.7 fold induction of PSA transcripts by DHT treatment at the 24 hour timepoint. But through the kinetic experiment, they saw that induction of PSA began between 4 and 6 hours post-treatment and they detected a 5 to 10 fold induction of PSA at 6 to 8 hours post-treatment. PSA mRNA levels subsequently declined, so that by the time samples were taken for SAGE analysis at 24 hours post-treatment, only a 1.7 fold induction was seen. The results of the clusterin kinetic experiment show an even greater effect of DHT treatment on induction and greater fluctuation ranges. Clusterin mRNA induction began sooner than PSA induction (only 0.5 to 1 hour post-treatment), declined between 6-12 hours post-treatment, and at the 24 hour timepoint clusterin mRNA levels had declined to a lower level than the untreated control cells. Thus, while clusterin mRNA was initially induced to much higher than steady-state levels by DHT treatment, by the time the researchers quantified the levels of clusterin mRNA with SAGE at the 24 hour timepoint, clusterin mRNA levels had fallen *below* the levels measured pre-treatment. Due the dynamic nature of these stimulation experiments, it is clear that the observed effect of DHT treatment on the mRNA level of an affected gene will depend on *when* the observation is made. For example, with clusterin, one could observe a large induction of transcription (1-6 hours post-treatment), no change in mRNA levels (some point between 12 and 24 hours post-treatment), or a reduction *below untreated levels* of mRNA (24 hours post-treatment), all depending on the particular timepoint chosen for the collection of an RNA sample from treated cells. Because of these fluctuations of mRNA levels over time, the data from Table 4 have no relevance to the relationship between steady-state levels of mRNA and protein for a particular gene and cannot inform us as to the general relationship between mRNA levels and protein levels. This is especially true since the authors did not perform kinetic experiments on proteins affected by DHT treatment; it is unknown whether reduced levels of expression seen for some proteins in the table represent a persistent suppression of protein expression over a 72 hour period or merely a reduced level at just the 72 hour timepoint. Thus, the data from Table 4, upon which the authors base their observation about the concordance of mRNA and protein levels, actually provide no insight into the relationship between mRNA levels and protein levels in a dynamic experiment with stimulated cells, let alone for cells in a steady-state environment.

The PTO has cited the observations of Waghray *et al.* regarding their experiments on stimulated cells in support of its argument that mRNA levels are not necessarily predictive of

protein levels, even when there are changes in the mRNA level. But because of the differences in transcript and protein detection efficiency and the dynamic nature of the stimulation experiments, no correlations between transcript and protein levels can be accurately drawn from the data presented. The conclusions of the authors have no relevance to and do not support the PTO's argument.

Waghray presents no more than 2 discernably differentially expressed mRNAs for which polypeptide levels were not similarly differential. However, based on Waghray's arbitrarily selected timepoints for measurement, there is no way to conclude that these 2 instances accurately reflect the relationship between mRNA and protein levels in the cell. As such, Waghray provides little or no basis to doubt Applicants' asserted utility.

The PTO also cites the work of Sagynaliev *et al.* to support its argument that mRNA levels are not predictive of protein levels, even when considering changes in mRNA levels. The Sagynaliev *et al.* reference is a review of scientific papers regarding gene expression in colorectal cancer (CRC) and describes an attempt by the writers to create a "data warehouse" combining the results of multiple researchers and laboratories into one database. The authors present statistics about how many genes have been found to be differentially expressed at the mRNA level versus at the protein level in CRC studies. The PTO points to these statistics as evidence of the discordance between mRNA and protein levels, noting that while 982 genes were found to be differentially expressed in human CRC by genome-wide transcriptomics technologies, only 177 have been confirmed using proteomics technologies.

The work of Sagynaliev *et al.*, however, does not support the PTO's argument. In their conclusions, the authors are not suggesting that mRNA levels, changing or otherwise, are not predictive of protein levels. Instead, they see the disagreement between different studies, laboratories and experimental techniques as evidence that there is a great need for standardization in this research field: "Thus, the development of standardized processes for collecting samples, storing, retrieving, and querying gene expression data obtained with different technologies is of central importance in translational research" (pg. 3066).

Far from supporting the PTO's argument, the research of Sagynaliev *et al.* actually provides a list of problems with the research in the field which serve to reduce the reproducability of the experiments and thus make conclusions drawn from comparison of

experimental results less reliable. Three of the problems listed by the authors serve to undermine the PTO's use of the data discussed *infra* in support of their argument. First, multiple factors can affect the outcome of a microarray experiment used to analyze a transcriptosome, including technical, instrumental, computational and interpretative factors. The authors found that when comparing different microarray experiments on CRC samples, only four of 185 genes selected behaved consistently on three array platforms and the agreement on the results from two brands of microarray was only about 30% (pg. 3077). Second, in proteomic studies, protein gels have well-known technological limitations, so that even under well-defined experimental conditions, 2-D PAGE analysis is "hampered by a significant variability" (pg. 3077). Third, because of "small sample size (number of patients), large number of variables examined at once, and absence of double or triple experiments (arrays and gels are expensive and samples are rare), statistical analysis is often *not valid*" (pg. 3077, emphasis added). Thus, reproducibility between transcriptosome analysis experiments or between proteosome analysis experiments is hampered by both the lack of technical standardization and the inherent variability of microarray and protein gel technologies. If the reproducibility of experimental results within particular areas of research is questionable, it is unlikely that conclusions drawn by comparing experiments between these research areas (e.g., examining *different* molecular populations) would be valid.

Furthermore, Sagynaliev provides no basis to conclude that differential mRNA levels are not typically reflected by differential levels of the encoded polypeptide. Sagynaliev reports and discusses consideration for assembling results generated from different methodologies. Sagynaliev's assembled data are not directed to the question of how differential mRNA levels influence polypeptide levels. Sagynaliev does not provide a single example in which differential mRNA levels for a particular gene were accompanied by unchanged, or oppositely differential, protein levels. Accordingly, Sagynaliev's findings cannot lead one to conclude that based on the assembled published data on CRC, differential mRNA levels do not lead to corresponding differential levels of the encoded polypeptide.

The PTO cites the studies of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* as allegedly supporting the contention that differential mRNA levels are not typically accompanied by corresponding differential protein levels. However, Nagaraja *et al.* and Sagynaliev *et al.* do not report findings contrary to Applicants asserted utility. There is not even a single example in

Nagaraja *et al.* or Sagynaliev *et al.* which shows that for a particular differentially expressed mRNA, polypeptide levels were not similarly differentially expressed. The "comprehensive study" of Waghray *et al.* reports 2 instances of discernably differentially expressed mRNAs in which the differential mRNA levels were not accompanied by similarly differential protein levels. However, the probative value of these 2 instances is questionable in view of the arbitrary time points selected for measuring dynamic mRNA and protein levels. In conclusion, the whole of the teachings of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* provide little or no evidence that would lead one skilled in the art to doubt Applicants' asserted utility.

Applicants' Evidence Establishes that a Change in mRNA Level for a Particular Gene Leads to Corresponding Change in the Level of the Encoded Protein

In support of the assertion that changes in mRNA are positively correlated to changes in protein levels, Applicants previously submitted a copy of a second Declaration by J. Christopher Grimaldi, a copy of the declaration of Paul Polakis, Ph.D., excerpts from the Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular Biology of the Cell (3rd ed. 1994) and (4th ed. 2002), excerpts from the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)), a reference by Zhigang *et al.*, World Journal of Surgical Oncology 2:13, 2004, and a reference by Meric *et al.*, Molecular Cancer Therapeutics, vol. 1, 971-979 (2002). The details of the teachings of these declarations and references, and how they support Applicants' asserted utility, are of record and will not be repeated here.

As discussed above, in addition to the above supporting references, Applicants also previously submitted references by Orntoft *et al.*, Wang *et al.*, Munaut *et al.*, Hui *et al.*, Khal *et al.*, Maruyama *et al.*, Caberlotto *et al.*, Mizrachi and Shemesh, Stein *et al.*, Guo and Xie. These studies are representative of numerous published studies which support Applicants' assertion that changes in mRNA level generally lead to corresponding changes in the level of the expressed protein. In addition, Applicants submitted an additional 70 references which support the correlation between changes in mRNA levels and changes in the levels of the encoded polypeptides. Applicants maintain that these references demonstrate that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true.

Furthermore, because the Haynes *et al.*, and Gygi *et al.* references cited by the Examiner relate to the correlation between the static level of mRNAs and proteins globally, *i.e.* across different genes, Applicants previously submitted references by Futcher *et al.,* Godbout *et al.*, Papotti *et al.*, Van der Wilt *et al.*, Grenback *et al.*, Shen *et al.*, and Fu *et al.,* which showed good correlation between static mRNA levels and the levels of the encoded polypeptides. In addition, Applicants previously submitted 26 additional references which also support Applicants' assertion in that the references report a correlation between the level of mRNA and corresponding protein, contrary to the assertion of the PTO that mRNA and protein levels are not correlated.

With respect to the previously submitted Exhibits in support of Applicants' position that changes in mRNA levels are correlated with changes in the levels of the encoded polypeptides, the PTO asserts that, with the exception of the Ornoft reference, Exhibits 4-15 are each directed to a single gene, or a small number of genes, and are not persuasive in view of comprehensive studies where significantly larger numbers of transcripts and proteins were examined. Similarly, with respect to Exhibits 17-24, the PTO maintains that, with the exception of the Futcher reference, these references are all directed to analysis of single genes, or a small group of genes. According to the PTO, these references do not demonstrate trends found across proteins in general. The PTO asserts that more comprehensive analyses like Haynes, Gygi, Chen et al. or Futcher more accurately describe general trends.

For the reasons cited above, the references of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* provide little or no evidence to draw conclusions about the relationship between differential mRNA levels and consequent protein levels. In contrast, the references in Applicants' Exhibits, many of which examined individually a large number of single genes or small groups of genes, provide evidence of the Applicants' position that mRNA levels and protein levels are correlated.

The PTO concedes that the Ornoft and Futcher references examine more than just a small group of genes, but the PTO dismisses these two references. Applicants' submit that Ornoft and Futcher provide sound basis for supporting the conclusion that differential mRNA levels are typically accompanied by differential levels of the encoded polypeptide, and that the

PTO cannot simply ignore the teachings of Applicants references in favor of the teachings of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.*

Applicants have shown above that the findings of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* cannot be relied upon to draw conclusions about the relationship between mRNA and protein levels. Applicants maintain that, while many of the references submitted as Exhibits examine a single gene, in total they provide well over 100 instances where changes in mRNA levels correspond to changes in levels of the encoded polypeptides. Accordingly, Applicants maintain that together these references provide a comprehensive analysis and are representative of the general trend that mRNA levels correlate with the levels of the encoded polypeptides.

In addition, Applicants submit herewith a copy of a second Declaration by Dr. Polakis (attached as Exhibit 1) that presents evidentiary data in Exhibit B. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' second Declaration says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." Accordingly, the second Declaration of Dr. Polakis further supports Applicants' position that, in general, differential mRNA expression correlates with differential expression of the encoded polypeptide.

Applicants also submit herewith a copy of a declaration by Randy Scott, Ph.D. (attached as Exhibit 2). Dr. Scott is an independent expert in the field of molecular diagnostics, with over 15 years of experience. He is the author of over 40 scientific publications in the fields of protein biology, gene discovery, and cancer, and is an inventor on several issued patents. His curriculum vitae is attached to the declaration. In paragraph 10 of his declaration, Dr. Scott states:

> One reason for the success and wide-spread use of the DNA microarray technique, which has led to the emergence of a new industry, is that generally there is a good correlation between mRNA levels determined by microarray analysis and expression levels of the translated protein. Although there are some exceptions on an individual gene basis, it has been a consensus in the scientific community that elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue. Therefore, diagnostic

> markers and drug candidates can be readily and efficiently screened and identified using this technique, without the need to directly measure individual protein expression levels. *Scott Declaration* at ¶10 (emphasis added).

Applicants submit the opinion of yet another expert in the field that changes in mRNA level for a particular protein in a given tissue generally lead to a corresponding change in the level of the encoded protein. Importantly, Dr. Scott also states that, contrary to the contentions of the PTO, diagnostic markers can be identified "without the need to directly measure individual protein expression levels." This opinion is supported by Dr. Scott's extensive experience in the field, as well as the fact that an entire industry has developed around technology to assess differential mRNA expression. As stated previously, there would be little reason to study changes in mRNA expression levels if those changes did not result in corresponding changes in the encoded protein levels.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew. *See in re Rinehart,* 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976); *In re Piasecki,* 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985). "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument." *In re Alton,* 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir. 1996), *quoting In re Oetiker,* 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992). Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an examiner." *Id.* at 1583. Applicants also respectfully draw the PTO's attention to the Utility Examination Guidelines which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." *66 Fed. Reg. 1098, Part IIB (2001).*

In summary, Applicants have provided the second Declaration of Paul Polakis and the Declaration of Dr. Scott, in addition to the declarations and references already of record which support Applicants' asserted utility, either directly or indirectly. These references support the assertion that in general, a change in mRNA expression level for a particular gene leads to a

corresponding change in the level of expression of the encoded protein. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions. However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*

In conclusion, Applicants submit that they have offered sufficient evidence to establish that it is more likely than not that one of skill in the art would believe that, because the PRO1069 mRNA is differentially expressed in kidney tumor, the PRO1069 polypeptide will likewise be differentially expressed in kidney tumor. This differential expression of the PRO1069 polypeptide makes the claimed antibodies useful as diagnostic tools for cancer, particularly kidney tumor.

Utility – Conclusion

Applicants remind the PTO that the evidence supporting utility does not need to be direct evidence, nor does it need to provide an exact correlation between the submitted evidence and the asserted utility. Instead, evidence which is "reasonably" correlated with the asserted utility is sufficient. *See Fujikawa*, 93 F.3d at 1565 ("a 'rigorous correlation' need not be shown in order to establish practical utility; 'reasonable correlation' suffices"); *Cross*, 753 F.2d at 1050 (same); *Nelson*, 626 F.2d at 857 (same). In addition, utility need only be shown to be "more likely than not true." *M.P.E.P.* at § 2107.02, part VII (2004). Considering the evidence as a whole in light of the relevant standards for establishing utility, Applicants have established at least one specific, substantial, and credible utility. In view of the above, Applicants respectfully request that the PTO reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Rejections under 35 U.S.C. § 112, first paragraph – Enablement

The PTO maintains its rejection of Claims 1-5 as lacking enablement. The PTO states that because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility, one skilled in the art would not know how to use the claimed invention.

Applicants submit that in the discussion of the 35 U.S.C. § 101 rejection above, Applicants have established a substantial, specific, and credible utility for the claimed antibodies. Applicants respectfully request that to the extent the enablement rejection is based on a lack of utility, the PTO reconsider and withdraw the enablement rejection under 35 U.S.C. §112, first paragraph.

Rejections under 35 U.S.C. § 102 and § 103 – Anticipation and Obviousness

Claims 1-2 and 4-5 remain rejected under 35 U.S.C. 102(a) on the assertion that they are anticipated by Lal et al. (WO 00/00610, 1/6/2000). According to the Examiner, Lal et al. teach a polypeptide (SEQ ID NO:35), which is identical to the polypeptide of SEQ ID NO:50 and antibodies that bind the polypeptide.

Applicants have previously submitted the Declaration of Goddard et al., originally submitted in related U.S. Patent Application Serial No. 10/063,555. The declaration establishes that the presently claimed subject matter was conceived prior to Lal's earliest priority date of June 26, 1998 and diligently reduced to practice thereafter. Applicants argued that therefore the cited reference is not available as prior art.

The PTO responds by saying that "the Declaration states that an experiment performed on June 13, 2000, in which primers were used to determine the expression level of DNA59211 (SEQ ID NO:49 encoding claimed SEQ ID NO:50) in various tumor samples...There is insufficient evidence of a recognition or appreciation of the claimed invention or a permanent idea of the complete and operable invention prior to the experiment performed in June 13, 2000." *Final Office Action* at 21.

Applicants' 1.131 Declaration demonstrates that the claimed subject matter, more particularly the polypeptide of SEQ ID NO: 50, was conceived by Applicants prior to January 6, 2000. Furthermore, as evidenced by the Declaration and accompanying exhibits, Applicants exhibited diligence in reducing the subject matter of the claims to practice by performing various

assays to confirm the function of the polypeptide. Specifically, the declaration states that the sequences of SEQ ID NOs:49 and 50 were first disclosed in U.S. Provisional Application 60/088740, filed June 10, 1998, as SEQ ID NOs:1-3, in Figures 1 and 2. Clearly, the polypeptide of SEQ ID NO:50 was conceived at least by the June 10, 1998 filing date of the provisional application as it is disclosed therein. In addition, variants of SEQ ID NO:50 and the remainder of the invention as presently claimed was also clearly conceived at least by the filing date of the provisional application, as a reading of the entire provisional application makes clear. For example, the provisional application states "[i]n another aspect, the invention concerns an isolated PRO1069 polypeptide, comprising an amino acid sequence having ... at least about 95% sequence identity to the sequence of amino acid residues 1 or about 17 to 89, inclusive of Figure 2 (SEQ ID NO:3)." *Prov. Appl. No. 60/088740* at 4. The provisional application also states "[i]n another aspect, the invention concerns a PRO1069 extracellular domain comprising an amino acid sequence having ... at least about 95% sequence identity to the sequence of amino acid residues 1 or about 17 to X of Figure 2 (SEQ ID NO:3), wherein X is any one of amino acid residues 32 to 41 of Figure 2 (SEQ ID NO:3)." *Id.* Also disclosed are the manufacture of antibodies to PRO1069, and their use to detect PRO1069 expression in specific tissue types. *See, e.g. Id.* at 26-32. Thus, contrary to the PTO's assertion, the provisional application clearly demonstrates recognition or appreciation of the claimed invention or a permanent idea of the complete and operable invention prior to the experiment performed in June 13, 2000.

Applicants have therefore provided a declaration showing prior invention of the claimed subject matter. The declaration establishes that the presently claimed subject matter was conceived prior to Lal's earliest priority date of June 26, 1998, and therefore also prior to the publication date of January 6, 2000, and diligently reduced to practice thereafter. Applicants have established prior invention by their 131 Declaration. This is sufficient to remove the cited reference as prior art.

Claims 1-2 and 4-5 are also rejected under 35 U.S.C. 102(e) on the assertion that they are anticipated by Walker et al. (U.S. Patent 6,277,574 B1, 4/9/1999). Claims 1-5 are rejected under 35 U.S.C. 103(a) as unpatentable over Walker et al. in view of Queen et al. The PTO asserts that Walker et al. discloses a polypeptide (SEQ ID NO:11) that is identical to the polypeptide of SEQ ID NO:50 and monoclonal antibodies and antibody fragments that specifically bind the

polypeptide.

The PTO concludes the remarks relating to Lal et al. are applicable here as well, rejecting the Declaration of Goddard et al. filed under 37 C.F.R. 1.131.

As stated above, the 1.131 Declaration establishes that the presently claimed subject matter was conceived prior to the April 9, 1999 date for Walker et al., and diligently reduced to practice thereafter. The cited provisional application clearly demonstrates recognition or appreciation of the claimed invention or a permanent idea of the complete and operable invention prior to the experiment performed in June 13, 2000. Applicants have therefore established prior invention by their 1.131 Declaration. This is sufficient to remove the cited reference as prior art.

Applicants therefore respectfully request withdrawal of the rejections under 35 U.S.C. §§ 102 and 103.

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

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